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POLYUNSATURATED HYDROCARBONS IN THE STABLE FLY[1]

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Abstract—Three triply-unsaturated hydrocarbons were identified from cuticular lipids of male and mixed-sex stable flies, *Stomoxys calcitrans*. The major compound, (Z,Z)-1,7,13-pentacosatriene, and two minor compounds, (Z,Z)-1,7,13-tetracosatriene and (Z,Z)-1,7,13-tricosatriene, were synthesized. Samples of male and female stable flies that differed in age, seasonality, geographic origin, rearing conditions of adults, and methods of extraction were analyzed for the presence of these triolefins. Females were found to have small quantities of the same C_{25} triolefin, which appeared to be identical to that in males. No evidence was seen for attraction of males or females to natural or synthetic triolefins.

Key Words—Stable fly, *Stomoxys calcitrans*, Diptera, Muscidae, hydrocarbons, olefins, (Z,Z)-1,7,13-pentacosatriene, (Z,Z)-1,7,13-tetracosatriene, (Z,Z)-1,7,13-tricosatriene.

INTRODUCTION

Muhammed et al. (1975) reported the presence of a sex attractant pheromone for males in whole-body extracts of the female stable fly, *Stomoxys calcitrans* L. The active materials appeared to be polyunsaturated hydrocarbons present in modest quantities in mature females. In tests with two- or four-port olfactometers, male flies responded to an airstream that passed downward over treated filter paper, then through screen cones, and into a chamber which held 20 (two-port olfactometer) or 40 males (four-port olfactometer). Flies that had moved onto or through the choice ports in 15 min were counted. Most activity was

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obtained in the hexane–ether (99 : 1) eluate from silica gel liquid chromatography (LC), when 23% of the mature males were attracted with 10 female equivalents, compared to 6% to the blank. Three polyolefins collected from this fraction by preparative gas chromatography (GC) had 23, 24, and 25 carbons and were attractive in olfactometer tests after GC determination of purity and quantitation. Males contained about four times more total polyolefin than females, including the major C_{25} components; no further identification work was reported (Muhammed, 1975).

In a companion report, Uebel et al. (1975), reported this class of compounds in male flies, but not in females isolated since eclosion. These workers identified and synthesized one component from male flies, (*Z,Z*)-1,7,13-pentacosatriene (Sonnet et al., 1977), and its geometric isomers were synthesized (Sonnet, 1979).

Owing to conflicting reports concerning the presence and quantity of C_{25} polyolefin in female flies, we reinvestigated the origin of this compound in male and female flies and its biological activity. The identification and synthesis of the 23-, 24-, and 25-carbon compounds found in stable fly polyolefins are described. The parameters considered in these tests were geographic location, seasonality, rearing conditions of adults, and methods of extraction.

METHODS AND MATERIALS

Biological Material. Flies used in this study were originally obtained from flies colonized at the Gainesville laboratory in 1976, 1977, and 1984 or from pupae collected in the wild at St. Croix, U.S. Virgin Islands in 1976. All adult flies were reared in cages as previously described (Muhammed et al., 1975).

The C_{25} polyolefin content of "summer" (reared in June 1976) and "winter" (reared in January 1977) flies of the Gainesville laboratory strain and the wild St. Croix flies (reared in March 1977) was determined.

In one series of tests, 500–1000 newly eclosed flies were sexed within 24 hr of emergence, and maintained under various conditions described below before extraction: (1) 24 hr: flies immediately frozen; (2) virgin: flies sexed within 24 hr of emergence, then maintained for 5 days with consexuals; (3) semiisolated: Females sealed in a small paper tub with the bottom and portions of the sides removed and covered with three layers of tube gauze and the tub placed within a larger cage containing an equal number of males for 5 days; (4) pupae: isolated in individual vials until eclosion, and the adults reared with consexuals for 5 days; (5) mixed sexes (mated): newly emerged flies were held together for 5 days, then sexed. Females in (1) through (4) were sexed again before extraction.

All bioassays were conducted using equipment and techniques as described by Muhammed et al. (1975).

Extraction. Crude extracts of the variously reared flies were made in two separate steps in the following manner: (1) Frozen flies were immersed in 50 ml hexane for 24 hr, and the solvent decanted through filter paper; this procedure was repeated once more, and the extracts combined as the initial cuticular wash. (2) The same flies were immersed in 50 ml hexane for 5 days before decanting; flies were then ground in a mortar and reimmersed in hexane overnight before decanting. Both extracts were combined as the final wash.

Spermathecae from 12 mature males (5–6 days old) were dissected in water, transferred to a glass vial, dried, and extracted with hexane for GC.

Isolation and Analysis. Extracts were fractionated on 2 × 45-cm columns of silica gel (60–200 mesh, Baker); saturated and unsaturated hydrocarbons eluted with 200 ml of hexane, polyunsaturated hydrocarbons with 200 ml of 1% ether in hexane, and other lipids with 100 ml each of 10 and 50% ether in hexane. The hydrocarbons were separated into paraffins and olefins on 2 × 45-cm columns packed with 20% silver nitrate impregnated silica gel (60–200 mesh, HI-FLOSIL-AG, Analabs). The purity of each of these classes of compounds, including the polyunsaturates from all rearing regimens, was checked by thin-layer chromatography (TLC) on silica gel plates impregnated with 20% silver nitrate (250-μm Uniplates, Analtech) and compared with paraffin and olefin standards. Quantification was done by temperature-programed GC on a Varian 2100 with glass columns (1.8 m × 2 mm ID) of 3% SE-30 or a Varian 3700 with a fused silica DB-1 column (15 m × 0.23 mm) (Figure 1, Table 1). Preparative GC samples were separated on an aluminum column (3 m × 4 mm ID) packed with 5% SE-30 in a Varian 1400 using a splitter.

Spectra were obtained on a Perkin Elmer model 221G infrared spectophotometer in solutions of CCl_4 and as films to determine double-bond configuration of compounds from polyolefin fractions and synthesized materials.

Sites of unsaturation of three polyolefins were determined by microozonolysis (of samples separated by preparative GC) and GC analysis of the resulting aldehyde and dialdehyde fragments after the method of Beroza and Bierl (1967). Natural III was hydrogenated over neutral palladium catalyst to confirm the absence of branching by electron impact mass spectra (EI-MS) using a Varian MAT CH5 mass spectrometer (GC-MS) via a 3.2 m × 2 mm ID glass column of 3% OV-1. Methane chemical ionization mass spectra (CI-MS) were obtained to determine the molecular weights of I, II, and III and the aldehyde ozonolysis fragments of III with a Finnigan 1015C GC-MS having 1.8 m × 2 mm stainless-steel columns of 5% SE-30 or 5% HI-EFF-1-BP. Methane CI-MS analyses were repeated in 1984 with a Finnigan 4000 EI/CI GC-MS system via a fused silica capillary (DB-1, 15 m × 0.23 mm).

Synthesis. The major triene III was synthesized by the alkylation of 1 mol of 1,7,13-tetradecatriyne (Farchan) with 1 mol of 1-bromoundecane, using *n*-butyllithium and THF–HEMPA as solvents (Figure 2). Silica gel chromatogra-

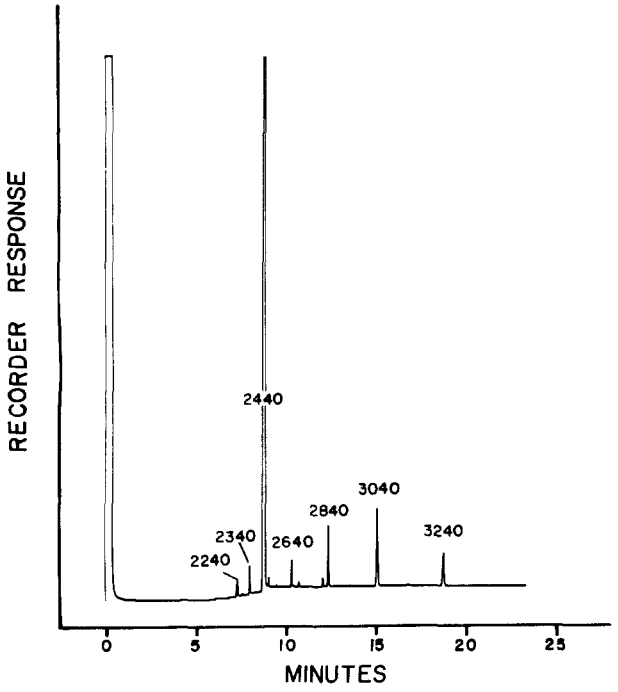

FIG. 1. Temperature programmed (200–300° at 12°/min) gas chromatogram of polyolefins from 1000 mixed sex stable flies on a fused silica DB-1 column (15 m × 0.23 mm ID), H_2 carrier gas.

phy of the monoalkylation product gave 60% yield of C_{25} triyne. Hydrogenation at 1 atm over 5% palladium catalyst poisoned with quinoline was followed by GC until the peak for the triene (KI2440) was maximized at 4 hr. The product, analyzed on silver nitrate TLC plates developed with 20% benzene in hexane, showed small amounts of *trans* compounds at R_f 0.7. The majority cochromatographed with the natural product, which also showed a smear at R_f 0.3–0.6. Silver nitrate chromatography on a 1 × 45-cm column gave a 60% yield of III from the triyne in the third fraction of 50% ether in hexane (100 ml), after hexane (150 ml) and 10% ether in hexane (100 ml) fractions has ben collected.

The mass spectrum of synthesized III was identical to that of natural III. Ozonolysis gave essentially the same aldehydes and dialdehydes by GC as natural III. A sample of III independently synthesized (Sonnet et al., 1977) was coincident by GC-MS and ozonolysis.

Homologs were prepared by addition of either 1-bromononane or 1-bromodecane to 1,7,13-tetradecatriyne followed by hydrogenation of the products as described to give (*Z*,*Z*)-1,7,13-tricosatriene (I) and (*Z*,*Z*)-1,7,13-tetracosa-

TABLE 1. PENTACOSATRIENE (III) FOUND IN EXTRACTS OF VARIOUSLY REARED 5-DAY-OLD STABLE FLIES[a]

Condition of flies[b]	No. flies	Micrograms found per fly	
		Initial wash[b]	Total[c]
Females			
24 hr	1028	0.015	
Virgin	668	0.085 (81%)	0.105
Semiisolated	553	0.048	
Isolated	309	0.007 (70%)	0.010
Mated	511	3.82	
Males			
24 hr	1094	0.020	
Virgin	539	8.30 (68%)	12.27
Semiisolated	449	10.96	
Isolated	254	2.46 (53%)	4.64
Mated	437	7.25	

[a] Determined by GC on 1.8 m × 2 mm ID glass column of 5% SE-30 on Gas Chrom Q (120-140 mesh).
[b] Refer to text for details.
[c] Combined extracts of the "initial wash" and "final wash" as described in the text.

triene (II). These compounds were separated on silver nitrate chromatography and were identical by ozonolysis, GC and CI-MS to natural I and II.

RESULTS

Identification. Retention indices for natural polyolefins were KI2240 (I), KI2340 (II), KI2440 (III), KI2640, KI2840, KI3040, and KI3240 (Kovats, 1965, Figure 1).

Infrared spectra showed vinyl (987, 902 cm^{-1}) and *cis* (739 cm^{-1}) adsorptions for the polyolefin fraction and for III after separation by preparative GC from I and II. The spectra obtained by methane CI-MS showed a small M strad-

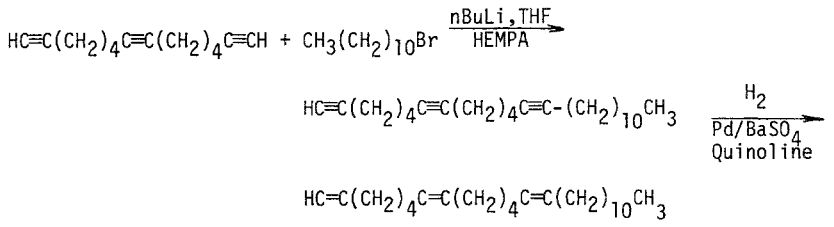

FIG. 2. Synthesis of 1,7,13-pentacosatriene (III).

dled by larger M − 1 and M + 1, characteristic for unsaturated hydrocarbons: I (*m/z* 317, 318, 319), II (*m/z* 331, 332, 333), and III (*m/z* 345, 346, 347). Thus, the molecular weights were 318, 332, and 346, respectively, with three double bonds in each.

Natural III was hydrogenated to a paraffin that eluted with *n*-pentacosane (KI2500) on an SE-30 column and showed a molecular ion at *m/z* 352 by EI-MS coincident with that of *n*-pentacosane.

Compounds that eluted later than III by GC, apparently in the same chemical class (triolefins), did not give visible parent ions with methane ionizing gas in early studies, and reconstructed ion chromatograms were not helpful. Later spectra obtained using isobutane CI-MS showed the presence of two C_{25} dienes (*m/z* 348) at KI2475 and 2465, and what was apparently a homologous series of diolefins including a C_{27} (*m/z* 374) at KI2655 and a C_{29} (*m/z* 402) at KI2855. These were present in an incompletely separated $AgNO_3$ column eluate of the triolefin fraction at about the same level as I and II (1–2%). Capillary CI/GC-MS of 1984 samples recovered from the lower half of the $AgNO_3$ TLC spot showed a clean pattern of homologous triolefins that appeared at KI2640 (0.2%, CI-MS mol. ion at *m/z* 374), 2840 (0.5%, *m/z* 402), 3040 (1.7%, *m/z* 430), and 3240 (0.5%, *m/z* 458) in 1984 mixed-sex flies.

These triolefins, quantified for each sex in 1984 (Table 2) were present in 1976 and 1983 colony flies in about the same quantities. In addition to III, recently studied (1984) males had more I and II than females, whereas mated females had ca. 5× more of the higher homologs that were also present in virgin females. Body parts from 1984 flies of both sexes were analyzed for III (Table 3), using the total hydrocarbon fraction, as III was well separated from other materials. Males and mated females had similar distribution of III, with the largest proportion on legs, and again the males had nearly 5 times more. In unmated females sexed within 18 hr, only abdomens contained III, and other

TABLE 2. POLYOLEFINS IN EXTRACTS OF STABLE FLIES (μg/fly)[a]

KI	Males (m, 4–5 days)[b]	females (m, 4–5 days)
2240	0.002	nd
2340	0.005	0.014
2440	11.19	0.048
2640	0.015	0.073
2840	0.010	0.083
3040	0.015	0.071
3240	0.010	0.046

[a] $AgNO_3$ TLC plate scrapes, 1984 flies; m = mated.
[b] From male abdomen sample × 5.328.

TABLE 3. PENTACOSATRIENE (III) IN-POOLED HYDROCARBONS FROM BLOOD-FED STABLE FLY BODY PARTS (μg/fly)

	Heads	Thoraces	Abdomens	Legs	Wings	Total
Females						
Unmated (3 days)[a]	0.0006	0.029	0.502	0.005	0.0015	0.54
Mated (4–5 days)[b]	0.032	0.510	0.529	1.192	0.320	2.58
Isolated (3 days)[c]	ND	ND	ND	ND	ND	
Males						
Mated (4–5 days)[b]	0.23	1.90	2.67	3.54	2.85	11.19

[a] Flies sexed within 18 hr of emergence ($N = 30$).
[b] $N = 30$.
[c] Virgin females emerged and reared in individual vials ($N = 8$).

body parts had only a trace. In contrast, III was not detected in a small sample ($N = 8$) of individually reared, isolated females, which agrees with Table 4.

Ozonolysis of the separated three major triolefins indicated that they formed a homologous series, as major cleavage fragments were decanal, undecanal, and dodecanal from I, II, and III, respectively (Table 5). These homologs were therefore, respectively, two and one methylene units ($-CH_2-$) shorter than III. Sites of unsaturation in all homologous triolefins appeared to be (1,7,13). Chromatograms of ozonolysis products of III were complicated by the presence of

TABLE 4. PENTACOSATRIENE (III) IN EXTRACTS OF STABLE FLIES FROM DIFFERENT LOCATIONS AND SEASONS[a]

Condition of flies[b]	Micrograms found per fly[c]
Females	
Gainesville summer	0.0543
Gainesville winter	0.0039
St. Croix March	>0.001
Males	
Gainesville summer	11.86
Gainesville winter	12.27
St. Croix March	14.89
Spermathecae[d]	none

[a] Determined by GC on 1.8 m × 2 mm ID glass column of 5% SE-30 on 124–140 mesh Gas Chrom Q.
[b] Sexed within 24 hr of eclosion, reared to 5 days old, ca. 1000 flies.
[c] Extracts combined as in "Total" in Table 1.
[d] Determined by capillary GC on 15 m × 0.23 mm DB-1 (trace of KI3000 observed).

TABLE 5. OZONOLYSIS FRAGMENTS OF NATURAL TRIOLEFINS DETECTED BY GC[a]

Aldehydes observed	Triolefin material ozonized (% composition)		
	C_{25}	C_{24}	C_{23}[b]
C_7	0		
C_8	0		
C_9	4.4		8.3
C_{10}	0	minor	79.2
C_{11}	1.1	major	
C_{12}	88.3		12.5
C_{13}	0.9		
C_{14}	0		
C_{15}	2.2		
C_{16}	3.1		
C_{17}	0		
C_{18}	0		
	100		100

[a] Analyzed on 3% SE-30 (1.8 m × 2 mm ID) glass column on 120–140 mesh Chromosorb W AW-DMCS.
[b] Analyzed on 5% Carbowax 20-M (1.8 m × 2 mm ID) s.s. column on 100–120 mesh Chromosorb W AW.

several dialdehydes. When a polar column (HI-EFF-1BP, modified DEGS) was used for CI-MS studies, the presence of large quantities of C_6 dialdehyde was confirmed. Dialdehydes prepared by ozonolysis of synthetic unsaturated aldehydes and alicyclic olefins were used as standards for comparison. Prominent ions seen in the methane CI-MS spectra of aldehydes were those for M + 1, M + 29, M + 41, M + 1 − 18, and M − 1 − 18, the first three ions resulting from the addition of H^+, $C_2H_5^+$ and $C_3H_5^+$, and the latter two ions due to loss of water. Dialdehydes showed additional prominent ions at M + 1 − 36 and M − 1 − 36 from loss of a water molecule from each end of the molecule. Distinction between mono- and dialdehydes was obtained by printing out the spectrum of each peak. Reconstructed mass chromatograms for the ions of M + 1 − 18 and M − 1 − 18 showed which compounds were monoaldehydes, as these peaks had no fragments at M + 1 − 36 or M − 1 − 36.

It appeared that III was chemically identical to the only polyene, (Z,Z)-1,7,13-pentacosatriene, described by Sonnet et al. (1977), as the major ozonolysis products, dodecanal and hexandial, were the same.

DISCUSSION

Quantitation of Polyolefins. Experments in repeated extraction showed that surface washes alone removed about 70% of III from either sex. Quantities

listed in Table 1–4 were not corrected for total extraction. At emergence, quantities of III were very low and roughly the same for males and females, but thereafter production in males greatly exceeded that in females. For flies reared in complete isolation, 5-day-old females produced only 0.2% as much III as males. A "primer" effect due to the presence of males was not responsible for the production of III in females, in the absence of direct physical contact, as shown by its absence in females that were semiisolated. Male and female flies reared in complete isolation produced 29 and 8% as much III, respectively, as those sexed at 24 hr and reared with consexuals as virgins. When flies were reared together, there was physical transfer of III from males to females. Note that the highest level of III was produced by semiisolated males which could see but not contact females. Harris et al. (1976) also reported some transfer of an unidentified C_{25} hydrocarbon (produced by mature males) to female flies during a single mating and more transfer of this material if females were held 24 hr with males.

Mature virgin males had similar quantities of III (12 μg, 95%) and small amounts of other polyolefins, regardless of their origin (Table 4), whereas the quantities of I and II were usually 0.15 μg (1.2%) and 0.26 μg (2.1%), respectively, and quantities of higher homologs were similar to I and II (Table 2). Virgin females had much smaller quantities of III, although those reared in summer had 12 times more III than those reared in winter in Gainesville (54 ng vs. 4 ng). We had expected to see a much larger increase in the level of polyolefins in virgin females with the onset of summer, but this was not observed. It must be noted that accidental inclusion of one male bearing 12 μg of III would raise the quantity in corresponding females by 4 ng/female and that extracts of 3000 winter-reared females had a total of 12 μg of C_{25} triolefin, the same quantity as one male. The flies were therefore carefully sexed two times, once at 24 hr and once after freezing. We found (fall 1976 and spring 1976) that the *trans* olefin and polyolefin content of female extracts were very small, as opposed to the quantities reported by Muhammed et al. (1975), and that no attraction was observed to any fractions with male flies in olfactometer tests. Bioassays of polyolefins and separated components recovered from females, mixed sex polyolefins, and three synthesized polyolefns described in this report, using the equipment and procedures of Muhammed et al. (1975) for attraction of males, gave negative results; also, no evidence was seen for biological activity of these materials in females (J.W. Mackley, unpublished data).

Females described as "unmated" (Table 3) are often described as "virgin" because they are not inseminated. However, these results suggest that physical contact is occurring since male-derived III appears exclusively on abdomens of "unmated" females that were sexed at 18 hr and held for 3 days. The mated females have spread III over all body parts, with proportions similar to those found in males. It is interesting to note that most was found on legs in older flies.

This process may be general in Diptera, as male tsetse flies of several species have been shown to transfer species-specific, male-exclusive long-chain alkenes to females of the same species upon mating (D.A. Carlson, unpublished data).

We feel that the differences in quantitation of III between our present results and those reported by Muhammed (1975) are the results of physical contact between sexes before the flies were knocked down at 18 or 24 hr for sexing. Less likely factors include careless sexing, seasonal changes (Table 4), loss of the original colony of flies at the University of Florida used by Muhammed (1975) that necessitated use of the USDA colony, a different strain being reared at a different location, or dietary changes involving the removal of antibiotic dosages from blood. Other factors that could have affected the strains differently include temperature and relative humidity of adult and larval maintenance, fly density in cages, larval rearing media, and methods of handling. The sex pheromone of a grass grub beetle (Scarabaeidae) has been directly linked to symbiotic bacteria (Hoyt et al., 1971), while Brand et al. (1975) observed bacterial conversion of α-pinene to *trans*-verbenol found in frass of a bark beetle (Scolytidae) *Ips paraconfusus*. House fly (*Musca domestica* L.) feces contain the hydrocarbon attractant (*Z*)-9-tricosene produced by cellular processes especially in mature female flies (Dillwith et al., 1981). However, it is possible that the presence of antibiotics in the blood diet of stable flies could cause decline in a bacterium-produced attractant. Preliminary tests to determine the effect of antibiotics on polyolefin production in females were inconclusive.

If the small quantities of polyolefin in extracts of females derived solely from males, it arrived in females through body contact before sexing at 24 hr. The female material should then be identical to male material, which should preclude its being a male attractant. If the triolefin fraction from the female contains an undiscovered sex attractant, it would have to be a uniquely potent material because there is such a small titer of any triolefin in mature virgin females. In any case, the minute quantity of C_{25} triolefin from females appeared to be chemically identical to that from males.

The proposition that male-produced mating deterrents against males are involved in the sexual communication schemes of several diptera is intriguing (Schlein et al., 1981). However, only one compound has been identified in Diptera that has an antiaphrodisiac function, as *cis*-vaccinyl acetate is produced by male *Drosophila melanogaster* and transferred to the female during mating (Jallon et al., 1981). We therefore investigated the components of the enlarged spermathecal duct of mature male stable flies and found small amounts of hydrocarbons in the crude extract, but no III.

Accessory-gland extracts from blood-fed male stable flies have been injected into females, and these receptivity-inhibiting substances prevented insemination at as low as 0.25 gland equivalents. The secretions were studied for their effect upon oviposition, fecundity and insemination in females. These receptiv-

ity-inhibiting substances were not found in sugar-fed males and were not identified (Morrison et al., 1982). However, we have no evidence that III is the responsible material.

CONCLUSION

Three triolefins identified from male stable flies were (Z,Z)-1,7,13-tricosatriene (I), (Z,Z)-1,7,13-tetracosatriene (II), and (Z,Z)-1,7,13-pentacosatriene (III). Molecular weights were obtained for several homologous trienes. Synthesized compounds (I–III) appeared to be identical to natural materials.

No evidence was obtained by bioassay to suggest that polyolefins observed in females or the synthesized compounds were attractive to males (Mackley, unpublished data). The small, but real, amount of (III) in unmated females appeared to be chemically identical to that present in males in much higher quantity, and small amounts of higher trienes were observed in both sexes. The differences in data in the tables are natural (i.e., variation among different batches of flies). The effects of seasonality, strain, "priming," geographic location, and age on the appearance of III in females are not significant enough to account for the large quantities reported in virgin females by Muhammed et al. (1975). We conclude that III found in virgin females in that study was derived from males before sperm transfer. Female stable flies do produce some III and other polyolefins, although the quantities found were small fractions (1/122 to 1/3000) of that produced by males upon maturation.

Alternatively, this material may be a primer used to stimulate the female to mate, as suggested by Harris et al. (1976), or an antiaphrodisiac that inhibits further mating with once mated females. We concluded that III is not transferred to females by genital contact alone in stable flies but that it is transferred by another route and that its function is unknown. However, the mechanism of transfer of large quantities of III to females is unknown, as is its biological origin. If a sex attractant is present in stable fly females, it does not appear likely that is a material described here.

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REFERENCES

BEROZA, M., and BIERL, B.A. 1967. Rapid determination of olefin position in organic compounds in the microgram range by ozonolysis and gas-chromatography. *Anal. Chem.* 39:1131-1135.

BRAND, J.M., BRACKE, J.W., MARKOVETZ, A.J., WOOD, D.L., and BROWNE, L.E. 1975. Production of verbenol pheromone by bacterium isolatad from bark beetles. *Nature* 254:136-137.

DILLWITH, J.W., BLOMQUIST, G.J., and NELSON, D.R. 1981. Biosynthesis of the hydrocarbon components of the sex pheromone of the housefly, *Musca domestica* L. *Insect Biochem.* 11:247–253.

HARRIS, R.L., OEHLER, D.D., and BERRY, I.L. 1976. Sex pheromone of the stable fly: Affect on cuticular hydrocarbons of age, sex, species, and mating. *Environ. Entomol.* 5:973–977.

HOYT, C.P., OSBORNE, G.O., and MULCOCK, A.P. 1971. Production of an insect sex attractant by symbiotic bacteria. *Nature* 230:472–473.

JALLON, J.M., ANTHONY, C., and BENAMAR, O. 1981. Un antiaphrodisiaque produit par les males *Drosophila melanogaster* et transferé aux femelles lors de la copulation. *C.R. Acad. Sci. Paris.* 292:1147–1149.

KOVATS, E. 1965. Pages 229-247, *in* J.C. Giddings and R.A. Keller (eds.). Advances in Chromatography, Vol. 1. Dekker, New York.

MORRISON, P.E., VENKATESH, K., and THOMPSON, B. 1982. The role of male accessory-gland substance on female reproduction with some observations of spermatogenesis in the stable fly. *J. Insect Physiol.* 28:607–614.

MUHAMMED, S. 1975. Stable fly (*Stomoxys calcitrans*) sex pheromones and their influence in males' sexual behavior. PhD thesis. University of Florida, Gainesville, Florida.

MUHAMMED, S., BUTLER, J.F, and CARLSON, D.A. 1975. Stable fly sex attractant and mating pheromones found in female body hydrocarbons. *J. Chem. Ecol.* 1:387–398.

SCHLEIN, Y., GALUN, R., and BEN-ELIAHU, M.N. 1981. Abstinons male produced deterrents of mating in flies. *J. Chem. Ecol.* 7:285–290.

SONNET, P.E. 1979. Synthesis of the male stable fly polyene (*Z,Z*)-1,7,13-pentacosatriene and its geometrical isomers. *J. Chem. Ecol.* 5:415–422.

SONNET, P.E., UEBEL, E.C., and MILLER, R.W. 1977. An unusual polyene from male stableflies, *J. Chem. Ecol.* 3:251–255.

UEBEL, E.C., SONNET, P.E., and MILLER, R.W. 1975. Sex pheromone of the stable fly: Isolation and preliminary identification of compounds that induce mating strike behavior. *J. Chem. Ecol.* 1:377–385.